Colonization of Extended-Spectrum- β -Lactamase-and NDM-1-Producing Enterobacteriaceae among Pregnant Women in the Community in a Low-Income Country: a Potential Reservoir for Transmission of Multiresistant Enterobacteriaceae to Neonates

Fanny Chereau, Perlinot Herindrainy, Benoit Garin, Bich-Tram Huynh, Frederique Randrianirina, Michael Padget, Patrice Piola, Didier Guillemot, Elisabeth Delarocque-Astagneau

▶ **To cite this version:**

Fanny Chereau, Perlinot Herindrainy, Benoit Garin, Bich-Tram Huynh, Frederique Randrianirina, et al.. Colonization of Extended-Spectrum- β -Lactamase-and NDM-1-Producing Enterobacteriaceae among Pregnant Women in the Community in a Low-Income Country: a Potential Reservoir for Transmission of Multiresistant Enterobacteriaceae to Neonates. Antimicrobial Agents and Chemotherapy, 2015, 59 (6), pp.3652-3655. 10.1128/AAC.00029-15 . hal-01292002

HAL Id: hal-01292002
https://hal.science/hal-01292002

Submitted on 22 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

AAC Journals.ASM.org

Colonization of Extended-Spectrum-β-Lactamase- and NDM-1-Producing *Enterobacteriaceae* among Pregnant Women in the Community in a Low-Income Country: a Potential Reservoir for Transmission of Multiresistant *Enterobacteriaceae* to Neonates

Fanny Chereau,[a,b,c] Perlinot Herindrainy,[d] Benoit Garin,[e] Bich-Tram Huynh,[a,b,c] Frederique Randrianirina,[f] Michael Padget,[a,b,c] Patrice Piola,[d] Didier Guillemot,[a,b,c,g] Elisabeth Delarocque-Astagneau[a,b,c]

Inserm UMR 1181, Biostatistics, Biomathematics, Pharmacoepidemiology and Infectious Diseases (B2PHI), Paris, France[a]; Institut Pasteur, UMR 1181, B2PHI, Paris, France[b]; University Versailles St-Quentin-en-Yvelines, UMR 1181, Biostatistics, B2PHI, Montigny-le-Bretonneux, France[c]; Epidemiology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar[d]; Laboratoire de Bactériologie Expérimentale, Institut Pasteur de Madagascar, Antananarivo, Madagascar[e]; Centre de Biologie Clinique, Institut Pasteur de Madagascar, Antananarivo, Madagascar[f]; AP-HP, Raymond Poincare Hospital, Garches, France[g]

The spread of extended-spectrum-β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) in low-income countries, where the burden of neonatal sepsis is high, may have a serious impact on neonatal mortality rates. Given the potential for mother-to-child transmission of multiresistant bacteria, this study investigated the ESBL-PE rectal colonization among pregnant women at delivery in the community in Madagascar and estimated a prevalence of 18.5% (95% confidence interval, 14.5% to 22.6%). One strain of *Klebsiella pneumoniae* isolated was also a New Delhi metallo-β-lactamase-1 (NDM-1) producer.

Severe bacterial infections are responsible for a large number of neonatal deaths in low-income countries (LICs) (1, 2), with *Enterobacteriaceae* most frequently isolated in neonatal sepsis (3, 4). The production of plasmid-borne extended-spectrum β-lactamases (ESBL) is the main mechanism of resistance against expanded-spectrum cephalosporins (ESC) among *Enterobacteriaceae*, with the CTX-M-15 variant being the most common enzyme (5). In LICs, where neonatal sepsis caused by ESBL-producing *Enterobacteriaceae* (ESBL-PE) may be associated with higher fatality rates (6), colonized mothers could represent a source of transmission to neonates. The objectives of this study were to estimate the prevalence of ESBL-PE rectal colonization among pregnant women in Madagascar in an urban area, Antananarivo, and a semirural area, Moramanga, and to identify associated risk factors.

TABLE 1 Characteristics of the participants in Madagascar, 2013 to 2014

Characteristic	Participant data[a]
Study area	
Antananarivo	149 (41.8)
Moramanga	207 (58.2)
Age (yr)	26 (21, 32)
Parity	1 (0, 2)
Marital status	
Single, divorced, or widow	18 (5.1)
Married or consensual union	338 (94.9)
Education	
Absent or primary school	98 (27.5)
Junior school graduate	184 (51.7)
High school or university graduate	74 (20.8)
Electricity access	
Yes	263 (73.9)
No	93 (26.1)

[a] The total no. of participants was 356. Data shown are median (interquartile range) or number (%).

This study was conducted in the context of an international pediatric cohort, the BIRDY (bacterial infections and antibiotic resistance disease among young children in low-income countries) program, approved by the Ethics Committees of Ministry of Health, Madagascar, and Institut Pasteur, France. All pregnant women living in the study areas were identified by medical or community workers and enrolled after giving written informed consent. A stool sample was taken at delivery for ESBL-PE screening. Among the women enrolled, 356 gave birth between June 2013 and March 2014 and were included in the present study. A risk factor analysis was conducted on a subset of 231 women who delivered during a 6-month period (June to September 2013 and January to March 2014). Sociodemographic characteristics, previous health care exposure, and antibiotic consumption (collected with prescription documents, remaining tablets, or visual aid) were recorded. Fresh stools were plated onto Drigalski agar supplemented with 3 mg/liter of ceftriaxone at the clinical laboratory of Institut Pasteur of Madagascar. Plates were incubated for 24 to 48 h at 37°C. Every oxidase and Gram-negative colony type was

Received 6 January 2015 **Returned for modification** 25 January 2015
Accepted 28 March 2015
Accepted manuscript posted online 6 April 2015
Citation Chereau F, Herindrainy P, Garin B, Huynh B-T, Randrianirina F, Padget M, Piola P, Guillemot D, Delarocque-Astagneau E. 2015. Colonization of extended-spectrum-β-lactamase- and NDM-1-producing *Enterobacteriaceae* among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant *Enterobacteriaceae* to neonates. Antimicrob Agents Chemother 59:3652–3655. doi:10.1128/AAC.00029-15.
Address correspondence to Fanny Chereau, fanny.chereau@inserm.fr.
F.C. and P.H. contributed equally to this article.
Copyright © 2015, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.00029-15

TABLE 2 Factors associated with ESBL-PE colonization among pregnant women in Madagascar, 2013 to 2014[a]

Factor	No. (%) of women by ESBL status		Univariate analysis[b]		Multivariate analysis	
	Positive ($n = 36$)	Negative ($n = 195$)	OR (95% CI)	*P*	OR (95% CI)	*P*
Study area						
Antananarivo	20 (55.6)	80 (41.0)	Ref			
Moramanga	16 (44.4)	115 (59.0)	0.6 (0.3–1.1)	0.11		
Age						
≤25 yr	13 (36.1)	109 (55.9)	Ref			
>25 yr	23 (63.9)	86 (44.1)	2.2 (1.1–4.7)	0.03		
Marital status						
Single, divorced, or widow	1 (2.8)	6 (3.1)	Ref	0.9		
Married or consensual union	35 (97.2)	189 (96.9)	1.1 (0.1–9.5)			
Education						
Absent or primary school	6 (16.7)	59 (30.3)	Ref			
Junior school graduate	17 (47.2)	98 (50.3)	1.7 (0.6–4.6)	0.29		
High school or university graduate	13 (36.1)	38 (19.5)	3.4 (1.2–9.6)	0.02		
Electricity access						
Yes	28 (77.8)	141 (72.3)	Ref			
No	8 (22.2)	54 (27.7)	0.7 (0.3–1.7)	0.49		
Birth attendant						
Unskilled worker[c]	5 (13.9)	62 (32.3)	Ref			
Skilled worker[d]	31 (86.1)	130 (67.7)	3.0 (1.1–8.0)	0.03		
Type of house						
Shared land use with other families	16 (44.4)	64 (32.8)	Ref		Ref	
Individual house	20 (55.6)	131 (67.2)	1.6 (0.8–3.4)	0.18	2.2 (1.0–4.8)	0.06
Drinking water supply						
Public tap	16 (44.4)	118 (60.5)	Ref		Ref	
Spring, well	13 (36.1)	65 (33.3)	1.5 (0.7–3.3)	0.34	2.2 (0.8–6.2)	0.15
Private inside access	7 (19.5)	12 (6.2)	4.3 (1.5–12.5)	0.01	3.8 (1.2–11.6)	0.02
Toilet facilities						
Shared with neighbors or public toilets	24 (66.7)	156 (80.0)	Ref			
Private	12 (33.3)	39 (20.0)	2.0 (0.9–4.3)	0.08		
Farmyard animals						
No	27 (75.0)	144 (73.8)	Ref			
Yes	9 (25.0)	51 (26.1)	0.9 (0.4–2.1)	0.89		
Poultry consumption						
<1/wk	24 (66.7)	130 (66.7)	Ref			
≥1/wk	12 (33.3)	65 (33.3)	1 (0.5–2.1)	1.00		
Hospitalization in the last year						
No	34 (94.4)	187 (95.9)	Ref			
Yes	2 (5.6)	8 (4.1)	1.4 (0.3–6.8)	0.69		
Close relative hospitalized						
No	23 (63.9)	156 (80.0)	Ref			
Yes	13 (36.1)	39 (20.0)	2.3 (1.0–4.9)	0.04		
Antibiotic treatment in the last year						
No	22 (61.1)	130 (66.7)	Ref			
Yes	14 (38.9)	65 (33.3)	1.3 (0.6–2.6)	0.52		
Antibiotic treatment in the last 3 mo						
No	29 (80.6)	165 (84.6)	Ref			
Yes	7 (19.4)	30 (15.4)	1.3 (0.5–3.3)	0.54		

[a] ESBL-PE, extended-spectrum-β-lactamase-producing *Enterobacteriaceae*.
[b] OR, odds ratio; 95% CI, 95% confidence interval; Ref, reference.
[c] Includes traditional birth attendants, nurses, or no attendant.
[d] Includes doctors and midwives.

identified with the API 20E system (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM). Inhibition diameters were read with the ADAGIO apparatus (Bio-Rad, Marnes-la-Coquette, France). Production of ESBL in ESC-resistant *Enterobacteriaceae* was confirmed by the double-disk synergy test (CASFM). When suitable, carbapenem MICs were obtained using Etest (bioMérieux, Marcy l'Etoile, France). Genomic DNA

was extracted with the GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). Plasmid-borne bla_{TEM} (7), bla_{SHV} (8), and bla_{CTX-M} β-lactamase genes (9) were screened by PCR. For ESBL-producing isolates also resistant to cefoxitin, the presence of bla_{AmpC} (10) and bla_{CMY-2} genes (11) were determined. ESBL-producing isolates showing a decreased susceptibility to carbapenems were screened for bla_{OXA-48} (12), bla_{KPC} (13), and bla_{NDM-1} (14) genes. All of the positive NDM-1 and half of the positive CTX-M PCR products were sequenced (Cogenics); for CTX-M-negative PCR results, SHV and TEM PCR products were sequenced. Statistical analyses were performed using Stata12 (Stata Corp., College Station, TX). All variables were analyzed by univariate logistic regression to identify factors associated with ESBL-PE colonization and, when associated with a *P* value of <0.2, were included in a multivariate model (manual backward logistic regression). Odds ratios (OR) with 95% confidence intervals (95% CI) were estimated, and the statistical significance was set at a *P* value of <0.05.

Among the 356 women included (Table 1), 66 were colonized with ESBL-PE, representing a prevalence of 18.5% (95% CI, 14.5% to 22.6%), with no significant difference between Antananarivo (22.1%) and Moramanga (15.9%) ($P = 0.14$) but a significant difference according to the delivery period. The prevalence was 9.6% between June and September 2013, 25.4% between October and December 2013, and 25.6% between January and March 2014 ($P = 0.001$ between the three periods). The 66 ESBL-producing isolates included *E. coli* ($n = 46$), *Klebsiella* spp. ($n = 11$), *Enterobacter cloacae* ($n = 5$), *Citrobacter freundii* ($n = 3$), and *Morganella morganii* ($n = 1$). Forty-five isolates carried a bla_{CTX-M} gene, 15 carried bla_{SHV} and bla_{CTX-M} genes, and 2 carried a bla_{SHV} gene. All of the sequenced genes were SHV-12, CTX-M-15, and non-ESBL TEM-1. Cefoxitin resistance was associated with bla_{AmpC} genes among 4 of these 62 ESBL-PE isolates: 1 *Enterobacter cloacae* (DHA), 1 *Citrobacter freundii* (CIT, FOX), 1 *E. coli* (MOX), and 1 *Morganella morganii* (DHA) isolate. The *E. coli*, *Citrobacter freundii*, and *Enterobacter cloacae* isolates also carried a bla_{CMY-2} gene. A bla_{NDM-1} gene was detected in a *Klebsiella pneumoniae* isolate exhibiting a reduced susceptibility to imipenem (MIC of 3 mg/liter). In 4 ESBL-producing *E. coli* isolates, no bla_{ESBL} gene, and no cefoxitin resistance, was detected. High susceptibility to fosfomycin (97%) and amikacin (100%) was found, while 36% of isolates were resistant to ciprofloxacin.

Of the 231 women interviewed, 79 had taken antibiotics during the previous year, and 22 received more than 1 antibiotic course. Most antibiotics belonged to the β-lactam family (79.4%). Thirty-six women were colonized with ESBL-PE (27 *E. coli*, 4 *Klebsiella* spp., 4 *Enterobacter cloacae*, 1 *Morganella morganii*). Several factors were identified in univariate analysis (Table 2); only private inside access to drinking water and living in an individual house remained associated with ESBL-PE colonization in the multivariate logistic regression model (adjusted for delivery period, June to September 2013 or January to March 2014, and study area). Antibiotic consumption was not significantly associated with ESBL-PE colonization.

Our estimation of the ESBL-PE colonization rate among pregnant women in the community (18.5%) is similar to those reported in Tanzania (15% in 2013) (15) and India (15% in 2007 to 2009) (16). A previous community-based study conducted in Madagascar in 2009 estimated an ESBL-PE carriage rate of 10% (17). Our results suggest an increase in the prevalence of colonization, consistent with the worldwide increase of ESBL-PE carriage in the community (18). Data on ESBL-PE colonization rates in LICs are scarce, especially among pregnant women, despite a risk of transmission from mothers to neonates (19–21). The isolation of an NDM-1-producing *K. pneumoniae*, the first NDM-1-PE identified in Madagascar, is of great concern. Importantly, the woman colonized with this strain did not report any recent hospital contact or antibiotherapy, suggesting a community acquisition. Living in an individual house and having a private inside access to drinking water were associated with ESBL-PE colonization. These conditions reflect a higher socioeconomic status in a globally underprivileged population and may correlate with more frequent access to health care services or a higher antibiotic consumption (16). However, we did not find any association between individual antibiotic consumption and ESBL-PE colonization. Due to the cross-sectional design of the study, the interpretation of the risk factors must be cautious. Finally, we observed a difference in the prevalence of ESBL-PE colonization according to the delivery period. The period from October to March corresponds with the rainy season in Madagascar, which may be associated with an increase of bacterial diarrhea and ESBL-PE fecooral transmission.

This study highlights a significant prevalence of ESBL-PE colonization among pregnant women in the community in an LIC and describes an NDM-1-PE maternal carriage, indicating that pregnant women may represent a substantial source for transmission of multiresistant *Enterobacteriaceae* to neonates.

ACKNOWLEDGMENTS

This work was supported by the Department of International Cooperation of the Principality of Monaco and by the French government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant ANR-10-LABX-62-IBEID). Fanny Chereau received a grant from Pierre Ledoux Foundation and a grant from Institut Pasteur International Division.

We thank all of the participating BIRDY study groups, physicians, laboratory staff, field interviewers, and community workers for their involvement in this project and the mothers for their cooperation during the data collection.

REFERENCES

1. **Seale AC, Blencowe H, Manu AA, Nair H, Bahl R, Qazi SA, Zaidi AK, Berkley JA, Cousens SN, Lawn JE.** 2014. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. Lancet Infect Dis **14:**731–741. http://dx.doi.org/10.1016/S1473-3099(14)70804-7.
2. **Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE.** 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet **379:**2151–2161. http://dx.doi.org/10.1016/S0140-6736(12)60560-1.
3. **Zaidi AKM, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA.** 2005. Hospital-acquired neonatal infections in developing countries. Lancet **365:**1175–1188. http://dx.doi.org/10.1016/S0140-6736(05)71881-X.
4. **Waters D, Jawad I, Ahmad A, Lukšić I, Nair H, Zgaga L, Theodoratou E, Rudan I, Zaidi AKM, Campbell H.** 2011. Aetiology of community-acquired neonatal sepsis in low and middle income countries. J Glob Health **1:**154–170.
5. **Bush K.** 2010. Alarming β-lactamase-mediated resistance in multidrug-resistant *Enterobacteriaceae*. Curr Opin Microbiol **13:**558–564. http://dx.doi.org/10.1016/j.mib.2010.09.006.
6. **Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE.** 2010. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatr **10:**39. http://dx.doi.org/10.1186/1471-2431-10-39.

7. **Weill F-X, Demartin M, Fabre L, Grimont PAD.** 2004. Extended-spectrum-beta-lactamase (TEM-52)-producing strains of *Salmonella enterica* of various serotypes isolated in France. J Clin Microbiol **42:**3359–3362. http://dx.doi.org/10.1128/JCM.42.7.3359-3362.2004.
8. **Arlet G, Rouveau M, Philippon A.** 1997. Substitution of alanine for aspartate at position 179 in the SHV-6 extended-spectrum beta-lactamase. FEMS Microbiol Lett **152:**163–167. http://dx.doi.org/10.1016/S0378-1097(97)00196-1.
9. **Bauernfeind A, Stemplinger I, Jungwirth R, Ernst S, Casellas JM.** 1996. Sequences of beta-lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other beta-lactamases. Antimicrob Agents Chemother **40:**509–513.
10. **Pérez-Pérez FJ, Hanson ND.** 2002. Detection of plasmid-mediated *AmpC* beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol **40:**2153–2162. http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002.
11. **Bauernfeind A, Stemplinger I, Jungwirth R, Giamarellou H.** 1996. Characterization of the plasmidic beta-lactamase CMY-2, which is responsible for cephamycin resistance. Antimicrob Agents Chemother **40:**221–224.
12. **Poirel L, Héritier C, Tolün V, Nordmann P.** 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother **48:**15–22. http://dx.doi.org/10.1128/AAC.48.1.15-22.2004.
13. **Queenan AM, Bush K.** 2007. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev **20:**440–458, table of contents. http://dx.doi.org/10.1128/CMR.00001-07.
14. **Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR.** 2011. How to detect NDM-1 producers. J Clin Microbiol **49:**718–721. http://dx.doi.org/10.1128/JCM.01773-10.
15. **Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, Zuechner A, Kihunrwa A, Mshana SE.** 2014. Evaluation of existence and transmission of extended-spectrum beta-lactamase-producing bacteria from postdelivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. BMC Res Notes **7:**279. http://dx.doi.org/10.1186/1756-0500-7-279.
16. **Pathak A, Chandran SP, Mahadik K, Macaden R, Lundborg CS.** 2013. Frequency and factors associated with carriage of multidrug-resistant commensal *Escherichia coli* among women attending antenatal clinics in central India. BMC Infect Dis **13:**199. http://dx.doi.org/10.1186/1471-2334-13-199.
17. **Herindrainy P, Randrianirina F, Ratovoson R, Ratsima Hariniana E, Buisson Y, Genel N, Decré D, Arlet G, Talarmin A, Richard V.** 2011. Rectal carriage of extended-spectrum beta-lactamase-producing Gram-negative bacilli in community settings in Madagascar. PLoS One **6:**e22738. http://dx.doi.org/10.1371/journal.pone.0022738.
18. **Woerther P-L, Burdet C, Chachaty E, Andremont A.** 2013. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev **26:**744–758. http://dx.doi.org/10.1128/CMR.00023-13.
19. **Chan GJ, Lee ACC, Baqui AH, Tan J, Black RE.** 2013. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med **10:**e1001502. http://dx.doi.org/10.1371/journal.pmed.1001502.
20. **Denkel LA, Schwab F, Kola A, Leistner R, Garten L, von Weizsäcker K, Geffers C, Gastmeier P, Piening B.** 2014. The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E). J Antimicrob Chemother **69:**2230–2237. http://dx.doi.org/10.1093/jac/dku097.
21. **Kothari C, Gaind R, Singh LC, Sinha A, Kumari V, Arya S, Chellani H, Saxena S, Deb M.** 2013. Community acquisition of β-lactamase-producing *Enterobacteriaceae* in neonatal gut. BMC Microbiol **13:**136. http://dx.doi.org/10.1186/1471-2180-13-136.